# ADAPTIVE RESPONSE OF CARP TO AQUATIC ENVIRONMENT CHANGES: A CASE STUDY

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#### Abstract

As part of a larger study, the present work highlights the way fish specimens affect the parameters of the aquatic environment, which is essential in fish farming. Six common carp (Cyprinus carpio) were subjected to an experiment where they were transferred to new aquatic environments, while two specimens remained in the water of origin. Each individual modified the initial values of the aquatic environments by balancing them to create a framework conducive to their survival. During the experiment, no feeding methods were applied, and the individuals' intervention on the water was strictly observed. The results demonstrated the high degree of adaptability of the species, but there was also an early case of fatality, which was justified. The intervention of the individuals was highlighted by the value of the parameter PO<sub>4</sub>, which exceeded the recommended value in aquaculture. However, the value was identical when each individual was removed from the experimental module.

Key words: aquatic environment, Cyprinus carpio, freshwater aquaculture, transfer.

## INTRODUCTION

Worldwide, increased attention is being paid to genetic studies of aquatic environments. Environmental DNA (eDNA) represents the trace that individual organisms leave in the environment they inhabit (Wilcox et al., 2013). Most genetic studies on aquatic macroorganisms have been based on water sample DNA (Minamoto et al., 2012; Miya et al., 2015; Ushio et al., 2017; Goldberg et al., 2018; Nelson-Chorney et al., 2019; Ishige et al., 2021), but other studies have focused their methods on underwater sediment DNA (Turner et al., 2015; Shaw et al., 2016; Valentini et al., 2016; Buxton et al., 2018). To date, a comparison of core properties between sample types has only been performed for fish DNA (Turner et al., 2015). Until now, the choice of applying one of the two methods (DNA extracted from the water sample or from the sediment) was aimed at accuracy and information only for fish DNA (Sakata et al., 2020) and only from environmental samples from seawater (Sutcliffe & Sharp, 1968; Maeda & Taga, 1973).

In the 1980s, specialists began sequencing DNA extracted from water and soil samples (e.g.,

Torsvik, 1980; Ogram et al., 1987; Bailiff & Karl, 1991). Microbial strains could not be cultivated in the laboratory, and researchers became interested in developing advanced techniques and technologies for their study (Henne et al., 2000; Michotey et al., 2013).

The intervention of fish in the aquatic environment is reflected in genetic material found in sediments and their composition (Wilcox et al., 2013), just as water parameters affect the genetic structure of the individuals that inhabit it (Markov et al., 2021). After describing the issue of adaptability, it is important to note that individuals are capable of adapting over time, typically through several generations (Witt & Huerta-Sanchez, 2019). However, problems arise when fish are manipulated and the environment of origin is changed in order to complete the technological process pursued within the economic flow.

In fish farms, water is considered the living environment of fish populations (Pilakouta et al., 2022), and it becomes an important and determining factor in the quality of fish products intended for human consumption. At the same time, for the economic yield of the farms, the quality-to-price ratio increases when there are no losses.

Significant losses occur during the transport of biological material from one farm to another without considering the parameters of the aquatic environments, both those of origin and those of transfer. In the case of reproduction, even minor differences between the aquatic environment of the fry and the aquatic environment in which it is transferred can result in losses of up to 80% of the number of specimens transferred, spontaneously or over time (Lostun et al., 2002).

# MATERIALS AND METHODS

## Materials

The aim of this study was to demonstrate the influence of individual fish on their aquatic environment, with a particular focus on the common carp (*Cyprinus carpio*).

The biological material used in the experiment consisted of eight C. carpio specimens obtained from three different farms, specifically selected based on the distance between them and the resulting differences in the chemical and biological aspects of their aquatic environments. The first farm, from which three scale-bearing C. carpio specimens were obtained, was the Moldavia Delta Complex (Larga Jijia) located at 47°21'14.7"N 27°22'12.4"E. This complex has been designated as a protected area of community interest by the Order of the Minister of Environment and Sustainable Development No. 1964/2007 on the establishment of the protected natural area regime of sites of community importance (Official Gazette No. 98, 2008). The complex consists of several lakes covering approximately 1250 hectares, where the common carp, Prussian carp, silver carp, and bighead carp are prevalent. As a polyculture farm, other species such as sheatfish, perch, and pike can also be found. The main activity of the farm is freshwater aquaculture, which includes not only fish growth but also reproduction. The farm also features a patented, scaleless variety of C. carpio known as topless Movileni carp.

The second farm from which 3 specimens of C. *carpio*, a variety with scales, were brought to the laboratory, was the Acvares Fish Farm at 47°19'23.8"N 27°32'06.4"E. It has an area of 237 hectares, of which 170 hectares is productive area. The farm produces fish from the species carp, including *Cyprinus carpio*, *Ctenopharyngodon idella*, *Hypophthalmichthys molitrix*, *Hypophthalmichthys nobilis*, *Silurus glanis*, *Sander lucioperca*, and *Polyodon spathula*, but the main species is common carp. The farm produces one summer fry (3 months old), two summer fry (15 months old), fish for consumption (1.5-2 kg), and selected breeder lines. Production and reproduction are carried out in ecological conditions and with the best quality feed, without the use of chemical fertilizers or manure.

The third farm from which 2 specimens of C. *carpio*, the scaled variety, were harvested for the study was Bârca Fish Farm at 47°04'40.1"N 27°30'05.7"E. It has an area of approximately 70 hectares of water divided into 2 ponds of 18 hectares at 47°04'39.1"N 27°30'04.1"E and 23 27°29'12.6"E. hectares at 47°04'40.9"N respectively, and 6 ponds for fry. The main activity of the farm is freshwater aquaculture, but it does not deal with the reproduction of fish material. The natural setting is preserved, and the feeding of the fry is carried out by the natural food created in the ponds during the cold seasons. The ponds are emptied and cleaned in the fall, after harvesting the saplings. Natural fertilizer is applied, represented by manure, and in the spring, the areas intended for the fish fry are flooded with water from the major accumulation.

The distance between the farms, as well as the technologies applied within each one, assured us that the fish material used in the study was healthy and resistant. The selection of individuals was carried out at the time of harvesting from each farm, following their transport and transfer under minimal stress conditions.

The experiment was carried out for 16 days, calculated from the release of the specimens in the studied environments until the last specimen showed signs of lethargy. The specimens of common carp (*Cyprinus carpio*) in the study were of the scaly variety, aged 18 months (one year and one summer), and presented the metric qualities detailed in Table 1.

Farm	Exemplary	Body mass (kg)	Total body length (cm)
A The Meldenie	Aı	2.00	36
A-The Moldavia	A2	1.98	35
Delta	A3	2.00	36
	Bı	2.10	37
<b>B</b> -Acvares	B2	2.00	36
	B3	2.10	37
C-Piscicola	C1	2.10	35
Bârca	$C_2$	2.30	36

 Table 1. Performance of the analysed characters in the specimens used in the study

### Methods

Samples were collected from each aquatic environment to establish the initial parameters of the water from which the specimens used in the experiment were collected (Table 2). The water was collected in a transparent glass container with a capacity of 1 liter, and the analyses were carried out at each farm during the collection of the biological material before its selection. To determine the parameters of interest, 1000 ml of water were collected and analysed from each aquatic environment. The parameters of interest included KH, GH, pH, NH4, NO2, NO3, PO4, SiO2, Fe, Cu, and O2, and their values were determined using a freshwater test laboratory called "JBL - Pro Aqua Test Lab".

After analysing the original aquatic environments, the individuals were distributed into different aquatic environments, some of which were new for the specimens analysed (Table 2).

 Table 2. Distribution of specimens in aquatic environments within the laboratory

Fish	Aquatic environment
1 1311	(100 l water)
Aı	
B <sub>2</sub>	M <sup>A</sup>
C1	
A2	
Bı	M <sup>B</sup>
C <sub>2</sub>	
A3	MP
B3	M

The experiment was conducted at a constant temperature of  $6^{\circ}$ C in the aquatic environments, and each individual was released into 100 liters of water. Since the specimens were at a constant temperature of  $6^{\circ}$ C, they had no appetite

(Official Gazette, Romania, 2008). In order to avoid any disturbances to the aquatic environment caused by external factors, including food, the influence of individuals on the environment was strictly monitored.

For aeration in each aquarium, Stream 480 submersible pumps with adjustable air flow were used, with the aim of minimizing the intervention on the aquatic environments. The pumps had a power of 4.3 W and a capacity of 520 L/h, which met the requirement for aeration in 100 L of water in each aquarium.

The data were statistically analysed, and the Tukey Test was used to determine the significance of the differences between the samples, whenever necessary, based on the results of the Fisher Test. The Tukey Test is a statistical test used for multiple comparisons between means (Tukey, 1949; Kramer, 1956). It is used to determine whether there is a significant difference between two or more means. The test is based on the Studentized Range Distribution and is also known as the Tukey-Kramer method or the Tukey HSD (Honestly Significant Difference) test.

The Tukey test is conducted after performing an analysis of variance (ANOVA) and if the F-test is significant. The formula for the Tukey test statistic is: q = (Yi - Yj) / SE, where q is the Tukey test statistic; Yi and Yj are the means being compared; and SE is the standard error, which is calculated as: SE = sqrt (MSW / n), where MSW is the mean square error from the ANOVA, and n is the number of observations.

The critical values for the Tukey test depend on the number of means being compared and the number of degrees of freedom. These critical values can be found in a table of the Studentized Range Distribution.

The Fisher Test, also known as Fisher's Exact Test, is a statistical test used to determine the significance of the association between two categorical variables (Fisher, 1922). It is used when the sample size is small, and the expected values are less than 5. The test is based on the hypergeometric distribution.

The formula for the Fisher test statistic is:

p = (r! \* (n1 - r)! \* (n2 - k)! \* (N - n1 - n2 + k)!) / (n1! \* n2! \* (N + 1)!), where:

p is the p-value;

r is the number of successes in sample 1; n1 is the sample size of sample 1; k is the number of successes in sample 2;

n2 is the sample size of sample 2;

N is the total sample size.

The critical value for the Fisher test can be found in a table of the Fisher's Exact Test distribution.

### **RESULTS AND DISCUSSIONS**

#### Results

The final parameters of the aquatic environments that were studied are listed in Table 3. The ", which represents the final values of the water parameters of the aquatic environment from A farm, were determined by  $A_1$ ,  $B_2$ , and  $C_1$  fish that were released into this water during the study. The " is represented by A<sub>2</sub>, B<sub>1</sub>, and C<sub>2</sub> fish that were released into B farm water at the end of the experiment. The next columns represent ", which shows the final values of the bottled still water that was populated by A<sub>3</sub> and B<sub>3</sub> fish, and the last column is for the aquatic environment of  $C_1$  and  $C_2$ carps, the water they originated from.

In the first case, the specimen that was released into the  $M^A$  water did not change the environment but was only retained in laboratory (aquarium) conditions. The change in parameters (Figure 1) was interpreted as a response to stress stimuli, with the individual being classified as wild. The reduction of the movement surface to 100 l of water represented a strong stress factor.



Figure 1. Average values of MA parameters with fish A1

The consumption of  $O_2$  shows the effort made by the specimen in adapting to the new conditions, resulting in changes to the KH and pH levels, without exceeding the recommended limits in aquaculture. The SiO<sub>2</sub> content decreases, falling within the maximum recommended limit of up to 1.2 mg/l, while PO<sub>4</sub> increases. Phosphorus, being the structural link in genetic material (DNA and RNA) and the component element of phospholipids in cell wall membranes, as well as in the structure of scales (Bud et al., 2010), marks the individual's intervention on the environment.

Aquatic environment		Fish			Fish		Fish		Мс			
Unit		A1	B <sub>2</sub>	C1		A <sub>2</sub>	Bı	C <sub>2</sub>		A3	B3	
KH(°dH)	14	25	23	26	18	27	28	30	9	12	21	19
GH(°dH)	15	14	13	14	22	20	25	21	9	11	11	12
pН	6.6	7.4	8	8.0	8	7.8	8	7.8	7.4	7.0	7.4	7.6
NH4(mg/L)	10	0.2	0.1	0.4	0.05	0.1	0.1	5	0.05	0.1	0.4	0.05
NO <sub>2</sub> (mg/L)	0.01	< 0.01	0.05	< 0.01	0.025	< 0.01	0.2	0.2	0.01	0.8	0.2	0.01
NO <sub>3</sub> (mg/L)	0.5	< 0.5	1	< 0.5	1	< 0.5	5	5	1	15	< 0.5	0.5
PO <sub>4</sub> (mg/L)	0.02	1.8	1.8	1.8	0.02	1.8	1.8	1.8	0.02	1.8	1.8	0.02
SiO <sub>2</sub> (mg/L)	3	0.8	0.2	0.8	0.1	< 0.1	0.2	0.2	0.1	3	0.2	0.1
Fe(mg/L)	0.05	0.05	< 0.02	0.05	1	0.05	< 0.02	0.1	0.02	< 0.02	0.05	0.8
Cu(mg/L)	0.05	< 0.05	< 0.05	< 0.05	0.05	< 0.05	< 0.05	< 0.05	0.05	< 0.05	< 0.05	0.05
O <sub>2</sub> (mg/L)	10	8	10	8	6	10	8	8	10	10	8	10

Table 3. Average values of the studied aquatic environments parameters

 $M^p$  - aquatic environment represented by bottled water;  $M^A$  - the aquatic environment from farm A;  $M^B$  - the aquatic environment from the specimens  $C_1$  and  $C_2$  originate; , and - stand for the initial values for the water from A and B farm and the bottled water.

Specimen A<sub>1</sub> was in M<sup>A</sup> water, its own aquatic environment, for 8 days, and at the first signs of lethargy (loss of balance, leaning to one side, etc.), it was removed from the studied aquatic environment and its parameters of interest were analysed (Tables 4 and 5). Specimen  $B_2$ , released into the aquatic environment  $M^A$ , in addition to the stress factor of retention under laboratory conditions, was also subjected to the stress caused by the modification of the

environment. The individual survived in the water from farm A for 11 days, intervening in the parameters of interest (Figure 2), balancing NH<sub>4</sub> and implicitly the nitrite and nitrate values. The individual consumed Fe, Cu and SiO<sub>2</sub>, balancing the values of these parameters. By comparing the values of  $M_i$  and  $MF^A(B_2)$ , we notice that B<sub>2</sub> brought the parameters closer to the values of its own aquatic environment, with major changes being recorded for KH (total hydrogen carbonate concentration), pH, NH<sub>4</sub> (ammonia), PO<sub>4</sub> (phosphate content), and SiO<sub>2</sub> (silicate content).

The KH value increased by 9 units, also exceeding the value of the environment of origin by 5 units. KH, together with carbonates and  $CO_2$ , form a buffer system that prevents fluctuations in the pH of the aquatic environment, and it can be seen that the pH has been adjusted to the pH value of the aquatic environment of origin, at 8.

The value of NH<sub>4</sub> decreased considerably, by 9.9 units, which is noteworthy, considering that ammonia has a high degree of toxicity for aquatic organisms (Bud et al., 2010). The power of the individual to reduce the value of NH<sub>4</sub> can be explained by the high degree of adaptability of the species, but which was enhanced by the activities carried out on the farm where it comes from, where natural methods of developing the biological material were applied.

PO4 (phosphate content), which has as its source "the general bio-geo-chemical circuit (mineralization of organic matter) including the processes of secretions, excretions, and cell lysis" (Bud et al., 2010), the other sources being irrelevant in laboratory (aquarium) conditions, increased up to 1.8 through the exchange of the specimen with the external environment. This fact emphasizes that the individual influenced the values of the water parameters, adjusting them according to his own needs.

Specimen  $B_2$  was studied for 11 days. Showing signs of lethargy, it was removed from the water, and the final parameters of the aquatic environment were analysed (Tables 4 and 5).

Following the changes recorded in the composition of the water from A farm, it can be seen that the parameters that underwent major changes are KH, NH<sub>4</sub>, PO<sub>4</sub>, and SiO<sub>2</sub>, while the other values were slightly influenced or not at all (Figure 3).



Figure 2. Average values of M<sup>A</sup> parameters with B<sub>2</sub> fish



Figure 3. Average values of M<sup>A</sup> parameters with C<sub>1</sub> fish

KH contributes to the formation of the buffer system that prevents fluctuations in the pH of the aquatic environment, and it can be seen that the pH did not exceed the maximum value up to which the carp develops within normal limits (7.7-9.00). However, it was modified by 1.4 units, exceeding the value of the water from which individual  $C_1$  originated.

The value of NH<sub>4</sub> also decreased considerably in this case by 9.06 units, having the same explanation as in the previous case.

Analysing the values of the parameters of the water from which specimen  $C_1$  comes, it is observed that the tendency of the changes made to the aquatic environment in which it was released is to bring the values as close as possible to those of its own environment. Thus, NO<sub>2</sub>, NO<sub>3</sub>, and Cu were brought to the values of the aquatic environment of origin.

The same  $PO_4$  content as in the cases of specimens  $A_1$  and  $B_2$  was also recorded here, appearing to be a standard modification applied by different individuals to the same environment.

As for the value of the  $O_2$  content, it was 2 units lower compared to the initial values of the aquatic environments studied, and of the aquatic environment of origin, but also of the aquatic environment in which it was released, demonstrating that the individual used a large amount of oxygen in the adaptability process. This fact is also supported by the aeration applied in the aquarium.

Specimen  $C_1$  spent 16 days in the aquatic environment  $M^A$ , modifying the aquatic environment parameters recorded in Tables 4 and 5. At the first signs of apathy, the individual was removed from the water, and the parameters of the water in which it was released during this period were analysed.

It is observed that the pH is perfectly balanced at the value of 7.8, the KH value has increased, helping to limit pH fluctuations (Figure 4). Coming from an environment with an NH4 content greater than 10 units, a slight increase in the level of this parameter is also observed in the aquatic environment where the specimen was released, and the increased value of phosphate, PO<sub>4</sub>, demonstrates the individual's intervention on the environment. It should be emphasized that the change in PO<sub>4</sub> was limited to the value of 1.8 units, as in the case of the previously analysed aquatic environments. The theory is increasingly taking shape that the intervention of a specimen in the environment in which it is released changes the phosphate value to this level.

In this case, the  $A_2$  specimen spent 9 days in the  $M^B$  environment, and at the first signs of apathy, it was removed from the water. The analysis of the water parameters in the aquatic environment M was carried out immediately after the extraction of the individual from the water (Tables 4 and 5).



Figure 4. Average values of M<sup>B</sup> parameters with A<sub>2</sub> fish

As in the previously presented situation of specimen A<sub>1</sub> that did not change the aquatic environment, in this case, also, the specimen that was released into water M<sup>B</sup>, was only retained in laboratory (aquarium) conditions. The change in parameters was also interpreted as a response to stress stimuli.

The pH remained unchanged,  $NH_4$  increased to the value of 0.1 mg/l, implicitly the values of  $NO_2$  and  $NO_3$  also increased (Figure 5), but the limits recommended in aquaculture were not exceeded.



Figure 5. Average values of M<sup>B</sup> parameters with B1 fish

It is observed the value of the phosphate content increased up to 1.8 mg/l as in the previous cases. Specimen B<sub>1</sub> was in the aquatic environment M<sup>B</sup>, its environment of origin, for a duration of 8 days, and at the first signs of lethargy, it was removed from the water and the parameters of interest were analysed (Tables 4 and 6).

Coming from the same environment as specimen C1, C2 showed the same tendencies to influence the water in which it was released. In the aquatic environment from B farm, having the values of the analysed parameters close to those of the parameters of the aquatic environment of the individual's origin, the O2 value increased by 2 units (Figure 6), showing that C<sub>2</sub> did not make as much effort to adapt as the specimen C1. However, the Fe content decreased from the value of 1 to 0.1, the optimal concentration in water is up to 1 mg/l (Bud et al., 2010), and the Cu parameter value stabilized below 0.05 mg/l, like that of the aquatic environment of origin, thus describing an intervention of the specimen on the new environment. Copper dissolved in water is readily absorbed by fish, but a concentration of copper sulfate greater than 0.8 mg/l can lead to chronic toxicity in numerous

species (Hepher, 1988; Barnabé, 1991; Macovei, 2008; Momeu et al., 2018).

KH was the parameter that increased its value the most, by 12 units, but contributing, together with carbonates and  $CO_2$ , to the creation of the buffer system that prevents pH fluctuations, the pH level fell perfectly between the value of the environment of origin and that of the new environment, stabilizing at 7.8, the specimen showing a high degree of adaptability.

The data is performed in Tables 4 and 6.



Figure 6. Average values of MB parameters with C2 fish

In this case, specimen A<sub>3</sub> was released in 100 l of still bottled water. Only 36 h after its release in the new environment, the individual presented physiological parameters incompatible with life, however, affecting the aquatic environment, significantly changing the values of NH<sub>4</sub>, NO<sub>2</sub>, NO<sub>3</sub>, PO<sub>4</sub> and SiO<sub>2</sub> (Figure 7).



Figure 7. Average values of MP parameters with A3 fish

Coming from the aquatic environment  $M_i^A$ , where the value of NH<sub>4</sub> is greater than 10 units, it is observed that, at the end of the experiment, the value of this parameter also increased in the water in which it was released. Implicitly, the NO<sub>2</sub> and NO<sub>3</sub> values also increased, the nitrite value being in the range of 0.5-1 mg/l, which describes, depending on the sensitivity of each species, fatal values. *Cyprinus carpio* is a species with a high degree of adaptability, but in the study, the value of 0.8 mg/l of nitrite proved to be fatal.

During the short period spent in bottled water, the specimen also affected the pH by balancing it between the pH value of the water from which it came,  $M_i^A$ , and the pH value of the bottled water in which it was released,  $M_i^P$ , namely at the value 7.

The value of the silicate content increased up to 3 units, equaling that of the aquatic environment of origin, and the phosphate value did not deviate from the rule observed so far in the experiment and increased to 1.8 units as in the previous cases.

The data is highlighted in Tables 4 and 6.

In this situation, specimen B<sub>3</sub> was released in 100 1 of still water. Coming from the  $M_i^B$  environment with values close to those of the water in which it was released, the individual spent 10 days in the new environment, during which time it intervened very little on the water parameters of interest.

However, it does not deviate from the rule that was observed in all specimens, increasing the phosphate value to 1.8 units. Coming from an environment with a water hardness higher than that of the new environment, B<sub>3</sub> contributed very little to the modification of NH<sub>4</sub> values, implicitly NO<sub>2</sub> and NO<sub>3</sub>, but also SiO<sub>2</sub>, Fe and Cu (Figure 8, Tables 4 and 7).



Figure 8. Average values of MP parameters with B3 fish

### Discussions

The released specimens in the water from A farm survived in the conditions created in the laboratory (in an aquarium with 100 l of water) for 8, 10, and 16 days, respectively. It is observed that the water hardness increased supporting the pH stability (Figures 1, 2, 3).

The initial value of the NH<sub>4</sub> content was over 10 units and, in each situation, the individuals managed to stabilize it at values much closer to those of the aquatic environments from which they came, except for specimen A<sub>1</sub>, which was not released in a new aquatic environment, but which brought significant changes to the water. The change in parameters was interpreted as a response to stress stimuli. The individual, due to the growth technologies applied in the farm and the extent of the accumulation surface from which it originates, is classified as wild, and the reduction of the movement surface to 100 l of water represented a strong stress factor.

The consumption of Fe, Cu and  $SiO_2$ , together with the fluctuations recorded in the  $O_2$  values, demonstrate the efforts made by each specimen to adapt, by creating the aquatic environment conducive to survival.

Under conditions created by the aquatic environment of B farm (Figures 4, 5, 6),  $B_1$  and  $C_2$  specimens brought more "bold" changes. Each individual deviated from the values of the parameters studied, without exceeding the limits recommended in aquaculture, and the phosphate content, as in the aquatic environment case of A farm, was fixed at the value of 1.8 mg/l by each individual, marking the degree of intervention on water.

In the aquatic environment represented by still bottled water (Figures 7, 8), specimen A<sub>3</sub>, which

came from water with NH<sub>4</sub> content value above 10 mg/l, survived only 36 h, bringing, however, changes to the aquatic environment in which it was released. The pH was balanced at the value of 7, and the value of the SiO<sub>2</sub> content increased to the value of the aquatic environment of origin, but the value of the nitrite content increased the toxicity of the water to the fatality of the individual.

Sample  $B_3$ , however, which came from an aquatic environment with values of water parameters close to those of still bottled water did not bring considerable changes to the water, but survived 10 days in the aquatic environment under study. The consumption of  $O_2$  demonstrates the effort made by the individual in the adaptability process.

Applying the standard statistical tests, it was possible to observe the differences between the aquatic environments and the influences brought to them by each fish. Thus, in Table 4 and Table 7 it can be observed that for the GH and Cu parameters, there were no influences of the analysed factor (i.e., the farm or the type of water) on the water quality. However, in the vast majority of cases, there were differences between the averages for the other parameters (such as NH<sub>4</sub>, phosphate, and SiO<sub>2</sub>) between the analysed groups. Thus, it can say that each fish brought significant changes to the water quality in the environment where it was released.

Aquatic environment	Ма		Мв			Мр		
Fish		р	C		р	C	٨	р
Parameter (unit)	A1	<b>D</b> 2	$C_1$	A2	<b>D</b> 1	C2	A3	<b>D</b> 3
KH (°dH)	***			***			***	
GH (°dH)		ns		***			***	
pH	***		***			***		
NH4 (mg/L)	***		***			***		
NO <sub>2</sub> (mg/L)	***		***			***		
NO <sub>3</sub> (mg/L)	***		***			***		
PO4 (mg/L)	***		***			***		
SiO <sub>2</sub> (mg/L)	***			**			***	
Fe (mg/L)	***			***			***	
Cu (mg/L)	ns			ns			ns	
O <sub>2</sub> (mg/L)	***			***			***	

Table 4. Fisher Tests for studied aquatic environments

\*\*\*  $\dot{F} > \dot{F}0.1\%$  - very significant differences between samples

\*\*  $\dot{F}1\% < \dot{F} < \dot{F}0.1\%$  - distinctly significant differences between samples

ns  $\dot{F} \leq \dot{F}5\%$  - insignificant differences between samples

Specification	- C1	- A1	- B2	B2- C1	A1- B2	A1- C1
KH (°dH)	***	***	***	**	ns	ns
pH	***	***	***	***	***	***
NH4 (mg/L)	***	***	***	***	***	***
NO <sub>2</sub> (mg/L)	***	ns	ns	***	ns	***
NO <sub>3</sub> (mg/L)	***	***	***	ns	ns	ns
PO <sub>4</sub> (mg/L)	***	***	***	ns	ns	ns
SiO <sub>2</sub> (mg/L)	***	***	***	***	***	ns
Fe (mg/L)	***	***	***	ns	ns	ns
O <sub>2</sub> (mg/L)	***	***	ns	***	***	ns

Table 5. Tukey Tests for A farm aquatic environment

\*\*\* w > w1% - very significant differences between samples \*\* w5% < w < w1% -significant differences between samples ns w < w5% - insignificant differences between samples

In the case of B farm aquatic environment, in addition to the parameters GH and Cu, it is also observed for other parameters such as  $SiO_2$  and

NH<sub>4</sub>, respectively, that there are no significant differences between the majority of the groups, as detailed in Table 6.

Specification	- A2	- B1	- C2	A2 - B1	A2 - C2	B1 - C2
KH (°dH)	***	***	***	ns	ns	***
pH	***	***	***	ns	***	***
NH4 (mg/L)	***	***	***	ns	ns	ns
NO <sub>2</sub> (mg/L)	***	***	***	***	*	***
NO <sub>3</sub> (mg/L)	***	***	ns	***	***	***
PO <sub>4</sub> (mg/L)	***	***	***	ns	ns	ns
SiO <sub>2</sub> (mg/L)	***	***	ns	ns	***	***
Fe (mg/L)	***	ns	***	**	***	***
O <sub>2</sub> (mg/L)	***	***	***	ns	***	***

Table 6. Tukey Tests for B farm aquatic environment

\*\*\* difference between averages > w1%, very significant differences between samples

\*\* w5% < difference between averages < w1%, significant differences between samples

ns difference between means < w5%, insignificant differences between samples

For bottled water, we notice that the factor influences water quality for almost all parameters, the only exception being Cu, as represented in Table 7.

Table 7. Tukey Tests for bottled water environment

Specification	- A3	- B3	A3- B3
KH (°dH)	***	***	***
GH (°dH)	***	***	***
pH	ns	ns	***
NH4 (mg/L)	ns	***	***
NO <sub>2</sub> (mg/L)	***	***	ns
NO <sub>3</sub> (mg/L)	***	ns	ns
PO <sub>4</sub> (mg/L)	***	***	ns
SiO <sub>2</sub> (mg/L)	***	ns	ns

\*\*\* difference between the averages > w1%, very significant differences between samples

\*\* w5% < difference between averages < w1%, significant differences between samples

ns difference between means < w5%, insignificant differences between samples

After applying the tests, the intervention of each individual in the aquatic environment that they inhabit is observed. These results are relevant in the context of good practices in fish farms, contributing to the application of good practices in aquaculture in order to optimize them economically.

### CONCLUSIONS

The results showed that the carp specimens were able to adapt to the new environment by bringing changes to the water parameters. The study found that NH4 (ammonia), PO4 (phosphate content), SiO<sub>2</sub> (silicate content), and KH (total hydrogen carbonate concentration) were the parameters that underwent significant changes due to the carp's intervention. The carp specimens were able to bring the parameters closer to the values of their own aquatic environment, which indicates their adaptability. The study also found that PO<sub>4</sub> was a standard modification applied by different individuals to the same environment. The study highlights the importance of monitoring the water parameters in aquaculture and how C. carpio's intervention affects these parameters.

The next research direction could focus on identifying the genetic and physiological factors that allow carp to adapt to new environments and intervene in the aquatic environment. Research could also explore the potential of carp to improve water quality and mitigate the impact of pollution on aquatic ecosystems. Another possible research direction could investigate the potential of carp's intervention in aquaculture for improving fish health and production.

#### REFERENCES

- Bailiff, M. D., & Karl, D. M. (1991). Dissolved and particulate DNA dynamics during a spring bloom in the Antarctic Peninsula region. Deep. Sea Res. Part A Oceanogr, Res. Pap., 38, 1077-1095.
- Barnabé, G. (1991). Base biologiques et écologiques de l'aquaculture. Paris, FR: Lavoiser - Tec et Doc. Paris Publishing House.
- Bud I., & Diaconescu St. (2010). Breeding of carp and other fish species. Bucuresti, RO: Ceres Publishing House.
- Buxton, A. S., Groombridge, J. J., & Griffiths, R. A. (2018). Seasonal variation in environmental DNA detection in sediment and water samples. PLoS ONE, e0191737. 13(1).
  - https://doi.org/10.1371/journal.pone.0191737

- Fisher, R. A. (1922). On the interpretation of  $\chi^2$  from contingency tables, and the calculation of P. Journal of the Royal Statistical Society, 85(1), 87-94.
- Goldberg, C. S., Strickler, K. M., & Fremier, A. K. (2018). Degradation and dispersion limit environmental DNA detection of rare amphibians in wetlands: Increasing efficacy of sampling designs. Science of The Total Environment. 633. 695-703. https://doi.org/10.1016/j.scitotenv.2018.02.295
- Nelson-Chorney, H. T., Davis, C. S., Poesch, M. S., Vinebrooke R. D., Carli, C. M., & Taylor, M. K. (2019). Environmental DNA in lake sediment reveals biogeography of native genetic diversity. Frontiers in Ecology and the Environment, 17(6), 313-318.
- Henne, A. S., Schmitz, R. A., Bömeke, M., Gottschalk, G., & Daniel, R. (2000). Screening of environmental DNA libraries for the presence of genes conferring lipolytic activity on Escherichia coli. Applied and Environmental Microbiology, 66(7), 3113-3116.
- Hepher, B. (1988). Nutrition of Pond Fishes. Cambridge, US: Cambridge University Press Publishing House.
- Ishige. T., Hara, H., Hirano, T., Kono, T., & Hanzawa, K. (2021). Genetic diversity of Japanese quail cathelicidins. Poultry Science, 100(5), 101046. https://doi.org/10.1016/j.psj.2021.101046
- Kramer, C. Y. (1956). Extension of multiple range tests to group means with unequal numbers of replications. Biometrics, 12(3), 307-310.
- Lostun, L., Turliu, N., & David, M. (2002). Ponds. Practical fish farming. Bucharest, RO: Ariesul Publishing House.
- Macovei, V. (2008). Researches into the usage of aquatic vegetation by several fish species [Doctoral thesis, University of Life Sciences "Ion Ionescu de la Brad"].
- Maeda, M., & Taga, N. (1973). Deoxyribonuclease activity in seawater and sediment. Marine Biology, 20, 58-63.
- Markov, D. A., Petrucco, L., Kist, A. M., & Portugues, R. (2021). A cerebellar internal model calibrates a feedback controller involved in sensorimotor control. Nature Communications, 12, 6694.
- Michotey, V., Méjean, V., & Bonin, P. (2000). Comparison of methods for quantification of cytochrome cd(1)-denitrifying bacteria in environmental marine samples. Applied and Environmental Microbiology, 66(4), 1564–1571. DOI: 10.1128/AEM.66.4.1564-1571.2000
- Minamoto, T., Yamanaka, H., Takahara, T., Honjo, M. N., & Kawabata, Z. (2012). Surveillance of fish species composition using environmental DNA. Limnology, 13, 193-197. https://doi.org/10.1007/s10201-011-0362-4
- of the Environment and Sustainable Ministry Development of Romania. (2008). Official Gazette no. 98 on February 7.
- Miya, M., Sato, Y., Fukunaga, T., Sado, T., Poulsen, J. Y., Sato, K.,... & Iwasaki, W. (2015). MiFish, a set of universal PCR primers for metabarcoding environmental DNA from fishes: Detection of more than 230 subtropical marine species. Royal Society Science, 2(7),150088. Onen https://doi.org/10.1098/rsos.150088

- Momeu, L., Cîmpean, M., & Battes, K. (2018). *Hydrobiology*. Cluj-Napoca, RO: Cluj University Press Publishing House.
- Ogram, A., Sayler, G. S., & Barkay, T. (1987). The extraction and purification of microbial DNA from sediments. *Journal of Microbiological Methods*, 7(2-3), 57–66. https://doi.org/10.1016/0167-7012(87)90025-X
- Pilakouta, N., O'Donnell, P. J., Crespel, A., Levet, M., Claireaux, M, Humble, J. L.,... & Parsons, K. J. (2022). A warmer environment can reduce sociability in an ectotherm. *Global Change Biology*, 29(1), 206-214. https://doi.org/10.1111/gcb.16451
- Sakata, M. K., Yamamoto, S., Gotoh, R. O., Miya, M., Yamanaka, H., & Minamoto, T. (2020). Sedimentary eDNA provides different information on timescale and fish species composition compared with aqueous eDNA. *Environmental DNA*, 2(4), 505–518.
- Shaw, J. L. A., Clarke, L. J., Wedderburn, S. D., Barnes, T. C., Weyrich, L. S., & Cooper, A. (2016). Comparison of environmental DNA metabarcoding and conventional fish survey methods in a river system. *Biological Conservation*, 197, 131–138. https://doi.org/10.1016/j.biocon.2016.03.010
- Sutcliffe, W. H., & Sharp, J. (1968). Measurement of deoxyribonucleic acid in the ocean and its ecological significance. *Limnology and Oceanography*, 13(3), 507–514. doi:10.4319/lo.1968.13.3.0507
- Torsvik, V. L. (1980). Isolation of bacterial DNA from soil. Soil Biology and Biochemistry, 12(1), 15–21. https://doi.org/10.1016/0038-0717(80)90097-8
- Tukey, J. W. (1949). Comparing individual means in the analysis of variance. *Biometrics*, 5(2), 99-114.

- Turner, C. R., Uy, K. L., & Everhart, R. C. (2015). Fish environmental DNA is more concentrated in aquatic sediments than surface water. *Biological Conservation*, 183, 93–102. https://doi.org/10.1016/j.biocon.2014.11.017
- Ushio, M., Murata, K., Sado, T., Nishiumi, I., Takeshita, M., Iwasaki, W., & Miya, M, (2017). Demonstration of the potential of environmental DNA as a tool for the detection of avian species. *Scientific Reports*, 8, 4493. https://doi.org/10.1038/s41598-018-22817-5
- Valentini, A., Taberlet, P., Miaud, C., Civade, R., Herder, J., Thomsen, P. F.,... & Dejean, T. (2016). Next-Generation Monitoring of Aquatic Biodiversity Using Environmental DNA Metabarcoding. *Molecular Ecology*, 25(4), 929–942. doi: 10.1111/mec.13428.
- Wei, N, Nakajima, F., & Tobino, T. (2018). A Microcosm Study of Surface Sediment Environmental DNA: Decay Observation, Abundance Estimation, and Fragment Length Comparison. *Environmental Science* & *Technology*, 52(21), 12428–12435.
- Wilcox, T. M., McKelvey, K. S., Young, M. K., Jane, S. F., Lowe, W. H., Whiteley, A. R., & Schwartz, M. K. (2013). Robust detection of rare species using environmental DNA: the importance of primer specificity. *PLoS ONE*, 8(3), e59520. https://doi.org/10.1371/journal.pone.0059520
- Witt, K. E., & Huerta-Sanchez, E. (2019). Convergent evolution in human and domesticate adaptation to high-altitude environments. *Philosophical Transactions of the Royal Society B*, 374, 20180235.